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Original communication

Immunohistochemical distribution of cyclic nucleotide phosphodiesterase (PDE) isoenzymes in the human vagina: A potential forensic value?

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Abstract

Objectives: Phosphodiesterase (PDE) isoenzymes are key proteins involved in the maintenance of the normal function of various tissues of the human body including those of the male and female urogenital tract. More recently, PDEs and their main substrates, cyclic GMP and cyclic AMP, have also been assumed to play a crucial role in the control of the human vagina. In order to elucidate the potential significance of phosphodiesterases as marker proteins in female genital organs, it was the aim of the present study to evaluate by means of immunohistochemistry the distribution of cGMP- and cAMP-PDE isoenzymes in specimens of the human vagina.
Methods: Conventional immunohistochemical techniques (double antibody technique, laser fluorescence microscopy) were applied to sections of the human vaginal wall in order to evaluate the presence of the PDE isoenzymes 1, 2, 3, 4, 5 and 10.
Results: Immunoreactivities (IR) specific for PDE1 (cAMP/cGMP-PDE, Ca^{2+}/Calmodulin-dependent), PDE2 (cAMP-PDE, cGMP-dependent) and PDE5 (cGMP-PDE) were exclusively registered in the smooth musculature of vaginal arterial vessels, whereas no signals were detected in non-vascular tissue. IR indicating the expression of the cAMP-degrading PDE4 was mainly observed in the vaginal epithelium. Vaginal epithelial cells also presented immunosignals specific for PDE3 (cAMP-PDE, inhibited by cGMP) and PDE10 (dual substrate PDE), nevertheless, these stainings were less abundant than those related to the PDE4. IR for PDE10 was also registered in inflammatory cells located in the subepithelial region of the vaginal wall.
Conclusion: Our study revealed the presence of IR specific for PDE1, PDE2, PDE4, PDE5 and PDE10 in sections of the human vagina and demonstrated that these enzymes are not evenly distributed in the tissue. Especially, the prominent expression of the cyclic AMP-PDE4A in the vaginal epithelium may give hint to a potential significance of this isoenzyme as a forensic marker protein. The findings give a rationale to investigate further as to whether the immunohistochemical detection of PDE4 may represent a new forensic tool in order to identify human vaginal epithelial cells.

Keywords: Phosphodiesterase isoenzymes; Vaginal epithelium; Sexual assault

1. Introduction

Up until now, numerous studies have reported on morphological markers considered useful in verifying if a cell

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cluster or tissue specimen originates from the female genital tract. Especially, since the pioneer work of Wiegmann and Merkel, the staining procedure according to Lugol has been widely used in forensic daily practice as a simple and reliable method in order to allow the identification of vaginal epithelial cells, and is, thus, routinely utilized in the investigation of cases of sexual assaults.[1,2] However, due to the fact that Lugol-reactive polygonal cells are not exclusively limited to female genital organs but also found in for example, the male distal urethra, the specificity of this technique has been critically discussed and the use of other potential tissue markers proposed.

To date, there is abundant evidence from the literature that the cyclic AMP- and cyclic GMP-signaling pathway and its key enzymes (nitric oxide synthases = NOS, guanylyl-and adenylyl cyclases = GC, AC, phosphodiesterases = PDEs, cyclic nucleotide-dependent protein kinases = PKA, PKG) are not only involved in the control of airway and gastrointestinal smooth muscle, the cardiovascular system and male penile erectile tissue but also the normal function of female genital organs including the vagina. With sexual stimulation, alterations in vaginal vascular and non-vascular smooth muscle tone occur, subsequently leading to an increase in the luminal diameter of the vagina, as well as in local blood flow and lubrication. It has been suggested that these events are mediated by an enhancement in cyclic nucleotide levels in the vaginal tissue. Cyclic AMP and cyclic GMP are synthesized from their corresponding nucleoside triphosphates ATP or GTP by the activity of adenylyl cyclases or guanylyl cyclases, respectively, and are degraded by cyclic nucleotide phosphodiesterases, a heterogenous group of hydrolytic enzymes. To date, 11 families of PDE isoenzymes have been identified, some of these families contain more than one gene (isogenes) and some genes are alternatively spliced. PDEs are classified according to their specificity for cyclic AMP or/and cyclic GMP, kinetic parameters of cyclic nucleotide hydrolysis, properties on anion exchange columns and sensitivity towards allosteric modulators of the enzyme activity.[3,4] It has been demonstrated that the distribution of PDE isoenzymes varies in the human body: While different tissues can be characterized by an almost identical pattern of PDE isoenzyme expression or the presence of at least more than one variant of an individual isoenzyme family, there are numerous examples where the expression of a PDE is tissue specific.[5,6]

In order to evaluate whether PDE proteins might be utilized as an additional morphological marker in female genital tissue, the aim of this study to elucidate the expression and distribution of some cAMP and cGMP-binding phosphodiesterase isoenzymes in sections of human vaginal tissue.

2. Materials and methods

2.1. Tissue sampling

Specimens of human vaginal tissue were obtained from five pre-menopausal female cadavers (aged 18–42 years) who had been subjected to standard autopsy. Full wall preparations were taken from the lower mid portion of the vagina within 6 h postmortem, immediately placed in an ice-cold solution of 4% formaldehyde in phosphate buffered saline (PBS, pH 7.4), and further processed as described below. All procedures were conducted in accordance with the regulations of the local ethical committee of the Hannover Medical School.

2.2. Immunohistochemistry

After immersion-fixation (4 h) in PBS with 4% formaldehyde, tissue preparations were rinsed several times with PBS containing 15% (w/w) sucrose and then embedded in Tissue-Tec (Miles Laboratories, Elkhart, IN, USA). Tissue specimens were sliced with a cryostat to sections of 8–10 μm thickness and thaw-mounted onto glass slides. Sections were pre-incubated (2 h) in PBS with 0.2% Triton X-100 and 0.1% BSA, followed by incubation for 24 h or 48 h with antibodies directed against PDE1A, PDE2A, PDE3A, PDE4, PDE5A and PDE10A diluted (1:250) in PBS with Triton X-100 (0.2%) and BSA (0.1%). After rinsing, sections were incubated for 90 min with respective fluorescein isothiocyanate (FITC)-(1:80), or Texas Red (TR)-(1:160) conjugated IgG antibodies. Hereafter, sections were mounted and visualized in an Olympus 3×50 fluorescence microscope (Olympus, Osaka, Japan).

2.3. Chemicals and antibodies

Antibodies directed against PDE isoenzymes were purchased from FabGennix Inc., (Shreveport, LA, USA). Texas Red (TR) and Fluorescein (FITC) dye conjugated immunoglobulins were obtained from Jackson Immunoresearch Laboratories Inc. (West Grove, PA, USA). Selectivity of the PDE antibodies was evaluated using Western blotting and ELISA protocols. The antibodies used in the study did not cross-react with other members of the PDE family.[7,8]

3. Results

3.1. Immunoreactivity (IR) for PDE isoenzymes

Immunoreactivities for all PDE isoenzymes, which were subject of the evaluation, were observed in the human vagina. Significant immunoreactive signals specific for the PDE isoenzymes 1A (cAMP/cGMP-PDE, Ca^{2+}/Calmodulin-dependent), 2 A (cAMP-PDE, stimulated by cGMP) and 5A (cGMP-PDE) were almost exclusively detected in vascular smooth muscle of the human vagina. Arterioles, interspersed in the subepithelial region, contained PDE1A- PDE2A- and PDE5A-immunoreactive smooth muscle cells (Figs. 1A–C). No immunosignals specific for PDE1A, PDE2A or PDE5A were observed in the vaginal epithelium. While only a minor degree of IR for PDE3A (cAMP-PDE, cGMP-sensitive) was registered in

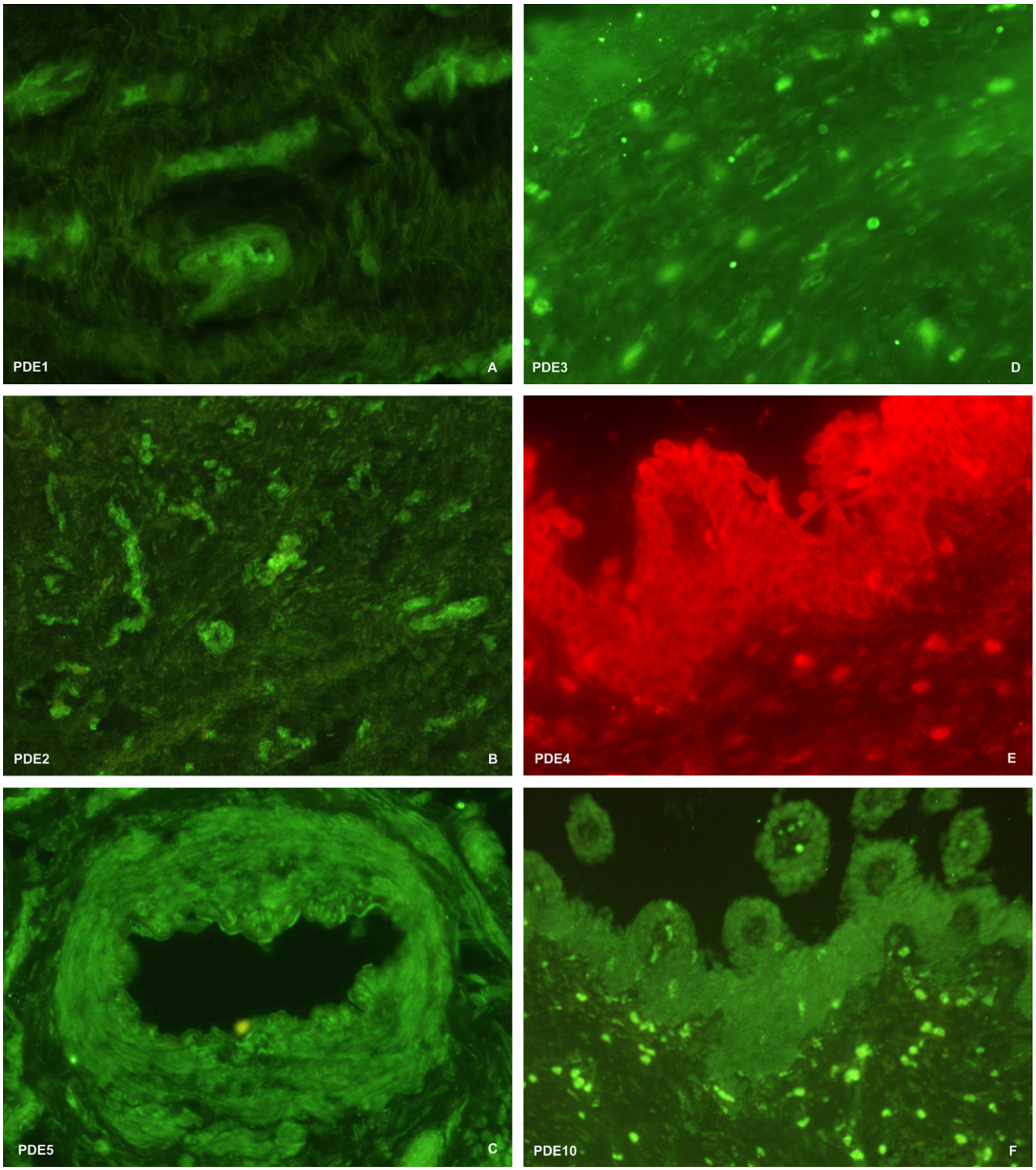

Fig. 1. Immunohistochemical detection of cyclic AMP and cyclic GMP-PDE isoenzymes in thin sections of the human vaginal wall using the double-antibody technique and laser fluorescence microscopy: Localization of IR (FITC) specific for PDE1A (A), PDE2A (B) and PDE5A (C) in the smooth musculature of small arterial vessels transversing the subepithelial area of the vaginal wall. (D) Thin section of a full wall specimen immunostained for PDE3 (FITC): Immunosignals are observed in single inflammatory cells located in the subepithelial area, whereas the epithelium appears almost unstained. (E) and (F): Micrographs displaying the distribution of PDE4 (E) and the Dual Substrate PDE10 (F) in the vaginal epithelial multilayer and in subepithelial locations supposed to be inflammatory cells, respectively. Magnification: 20×.

the vaginal epithelial layer, single inflammatory cells located in the subepithelial space presented dense staining (Fig. 1D). No signals indicating the expression of PDE3 were detected in the smooth musculature of vaginal vessels. Investigation of the distribution of the cAMP-PDE4 revealed abundant IR in the vaginal epithelial mul-

tilayer. Epithelial cells exhibited strong immunofluorescence signals indicating the expression of PDE4 (Fig. 1E). IR for PDE4 was also observed in the smooth muscle portion of the vaginal wall as well as in an irregular meshwork of arteries supplying the tissue (data not shown). Expression of the dual substrate PDE10 was most prominent in the smooth muscle layer of the vaginal wall, whereas only weak immunoreactive signals were observed in the epithelium (Fig. 1F).

4. Discussion

For decades, standardized forensic methods have been applied in order to investigate in cases of sexual assaults on females. Usually, the examination includes swab specimens taken from the vagina and the penile surface. Glycogen-containing squamous epithelial cells, identified by means of the Lugol staining method, have long been considered a specific histological vaginal marker. Where these cells can be detected on the surface of a male penis, this is considered evidence that vaginal penetration had occurred. However, it has been demonstrated that Lugol-positive polygonal, glycogen-containing cells are also found in swab specimens taken from the oral cavity, male urethra and glans penis[9,10]. Thus, it has been suggested that there is a need to introduce new techniques, utilising molecular biology as well as the immunohistochemical detection of estrogen receptors, to allow accurate identification of vaginal epithelial cells.[11,12]

Cyclic nucleotide phosphodiesterases (PDEs) belong to a large and heterogenous family of hydrolytic enzymes, not only characterized by different biochemical and molecular genetic properties but also their distribution and regulatory significance in various tissues of the human body. PDEs play a key role in the control of multiple organ and tissue functions mediated by intracellular cyclic nucleotide-dependent signaling pathways. There is evidence from basic research that cyclic nucleotides and PDEs are involved in the maintenance of the normal function of the human vagina and clitoris, thus providing the physiological basis for an adequate genital response to sexual stimulation, subsequently leading to sexual arousal and orgasm.[13–15]

The results from the present study demonstrate that various cyclic AMP- and cyclic GMP-PDE isoenzymes are expressed in human vaginal tissue and that these isoenzymes are not evenly distributed. Our findings indicate that PDE1, PDE2 and PDE5 are almost exclusively located in vascular smooth muscle of the vagina and cannot be detected in the epithelial layer. The homogenous occurrence of the said PDEs in the smooth muscle component of vaginal arteries is in support of a significance of the enzymes in the control of blood flow to the vagina. In addition, the localization of PDE1, PDE2 and PDE5-IR smooth muscle cells in the network of arterioles extending towards the epithelium suggests a function for the isoenzymes in the regulation of vaginal fluid transudation. While IR specific for PDE5 in vascular smooth muscle cells of the human vagina has never been shown before, in contrast to results presented by D'Amati et al., we were unable to detect PDE5-IR in the epithelial layer of the vaginal wall.[16] The distribution of immunoactivity for PDE10 in the human vagina suggests that this isoenzyme is not of importance in the regulation of the tone of vascular smooth muscle, but may have a function in the mediation of immunological responses in the vaginal tissue. With regard to the distribution of the cAMP-PDE4 it is notable that none of the other PDE isoenzymes we investigated in our study were found to be present in abundant quantities in the cells of the vaginal epithelial multilayer.

In order to verify the occurrence of vaginal penetration, the forensic investigation of sexual assaults on females includes the attempt to recover vaginal polygonal cells from the surface of the potential assassin's penis. From the forensic point of view, the abundant expression of the cAMP-PDE4 in the vaginal epithelium gives hint to the speculation that this isoenzyme might represent an immunohistochemical marker for the identification of vaginal squamous cells. Although their results have not yet been confirmed, the feasibility of identifying PDE isoenzymes in exfoliated cells of the vaginal epithelium by means of immunohistochemistry has been demonstrated earlier by D'Amati et al. who reported the cytoplasmatic expression of the cGMP-PDE5 in scrap specimens harvested by gentle scratching of the anterior vaginal wall.[16] In addition, Chujor et al. demonstrated the differential expression of subtypes of PDE4 in cells harvested from another surface tissue layer of the human body, the epidermis. Based on related findings made by other groups, a potential significance of cyclic AMP phosphodiesterases in the pathophysiology of cutaneous inflammatory and allergic disorders, such as atopic dermatitis, has been proposed.[17–19] The results on the distribution of PDE4 in the human vaginal epithelium raise the question as to whether these findings can also be confirmed on the messenger RNA level. Up until now, knowledge on the expression of mRNA transcripts encoding for PDE isoenzymes in female genital tissues is few and far between. Only a single study using RT-PCR and the TaqMan protocol reported the detection of mRNA encoding for the PDE isoenzymes 1, 2, 4, 5, 10, and 11 in the human vagina. Interestingly, the authors presented evidence for a predominant expression of PDE1A and 4A in vaginal the epithelial layer.[20]

In conclusion, the non-homogenous distribution of PDE isoenzymes, especially the cAMP-PDE4, in thin sections of the human vaginal wall, which we observed in our study, provides rationale to explore further as to whether there is a value of PDE4 as an immunohistochemical vaginal epithelial marker protein in forensic science. Such studies should also include specimens from the male penile epidermis and glans penis, as well as from the oral and rectal mucosa of male and female individuals.

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